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### Pim1 kinase: a double-edged sword

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*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2015

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

de Vries, M. (2015). *Pim1 kinase: a double-edged sword: The divergent roles of a survival kinase in environment-airway epithelium interaction*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.

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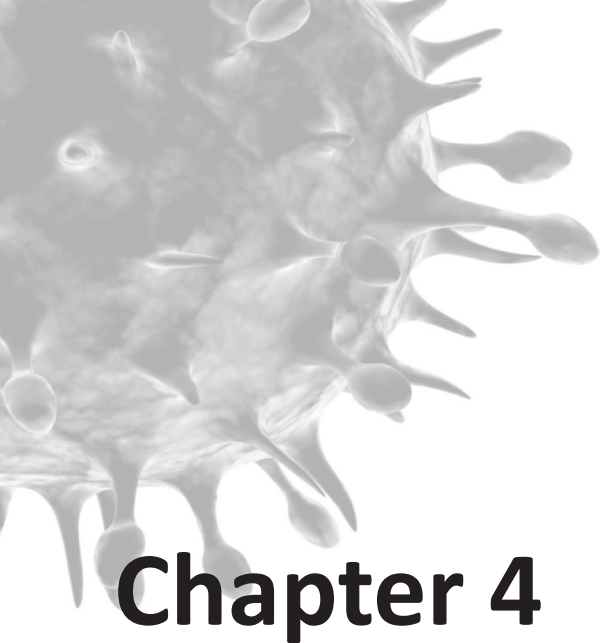
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# Chapter 4

***Inhibition of Pim1 kinase, new therapeutic approach  
in virus-induced asthma exacerbations.***

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and Donna E. Davies

## **Abstract**

Therapeutic options to treat virus-induced asthma exacerbation are limited and urgently needed. Therefore, we tested Pim1 kinase as potential therapeutic target in human Rhinovirus (HRV) infections. We hypothesized that inhibition of Pim1 kinase reduces HRV replication by augmenting the interferon-induced anti-viral response due to increased activity of the JAK-STAT pathway.

Air-liquid interface (ALI) cultures of primary bronchial epithelial cells (PBECS) from healthy individuals and severe asthmatic volunteers were infected with HRV-16 with or without a specific Pim1 inhibitor; viral replication and induction of anti-viral responses were measured using RT-qPCR. Viral titers were measured by TCID<sub>50</sub> and release of IP-10 and RANTES protein assessed by ELISA. Phosphorylation of STAT-1 was determined using western blotting.

Viral replication was reduced in ALI cultures of healthy and asthmatic PBECS treated with the Pim1 inhibitor. Using cultures from healthy donors, enhanced STAT-1 phosphorylation upon inhibition of Pim1 kinase activity resulted in increased mRNA expression of IFN- $\beta$ , IL-29, IP-10 and RANTES 12 hours after infection and increased protein levels of IP-10 and RANTES 24 hours after infection.

We have identified Pim1 kinase as novel target to reduce viral replication in ALI cultures of PBECS. This may open new avenues for therapeutic interventions in virus-induced asthma exacerbations.

## **Key words**

Rhinovirus; viral respiratory infection; airway epithelial cells; ALI; asthma; cytokine

## **Introduction**

Asthma is a chronic respiratory disease, affecting up to 10 percent of the population in the Western world [1]. It is characterized by reversible airflow obstruction, airway inflammation and airway hyper responsiveness, and is further complicated by frequent occurrence of exacerbations [1][2]. The main triggers of asthma exacerbations are infections with respiratory viruses, most notably human rhinoviruses (HRVs), and it has been shown that patients with asthma are more susceptible towards HRV infections compared to non-asthmatic individuals [3]. Asthma exacerbations are a major economic and social burden and therapeutic options for the treatment of virus-induced asthma exacerbations are limited to date, emphasizing the pressing need for novel therapeutics [1].

In the lower airways, the principal target cells for infection with HRV are bronchial epithelial cells. These cells form the first line of defense against potential harmful pathogens by constituting a physical and immunological barrier covering the entire surface of the respiratory tract [1][4][5]. HRV infection of epithelial cells induces the expression of the Type I and III interferons IFN- $\beta$  and IL-29, respectively, which in turn can activate the JAK-STAT signaling pathway [4][6][7][8]. Subsequent transcription of a wide range of interferon inducible genes results in reduced viral replication and limitation of viral spread [6]. It has been shown that patients with asthma display an impaired interferon response upon infection with HRV resulting in increased viral replication compared to healthy individuals [9][10]. Notwithstanding, primary bronchial epithelial cells of asthmatic volunteers exhibit a normal cellular response to IFN- $\beta$ , and exogenous administration of IFN- $\beta$  has been postulated as a viable therapeutic treatment in virally induced asthma exacerbations [11]. Indeed, recent clinical trials have shown beneficial effects of inhaled IFN- $\beta$  in viral-induced exacerbations in patients with difficult-to-treat asthma [12]. These findings support the concept of identifying approaches that augment the induction of an interferon-induced anti-viral response as novel therapeutic strategy.

The interferon activated JAK-STAT signaling pathway is negatively regulated by suppressor of cytokine signaling (SOCS) proteins [13] [14]. Expression of SOCS-1 and SOCS-3 was found to be increased by HRV infection of PBECs in a TGF $\beta$ -dependent fashion [15], suggesting a contribution of SOCS proteins to desensitization of HRV infected PBECs to Type I interferon responses. SOCS proteins can be stabilized by the Pim family of serine/threonine kinases, leading to further reduction of JAK-STAT signaling [16]. Pim kinases are involved in a wide range of cellular processes like cell differentiation, cell survival and cytokine signaling and we have previously shown that Pim1 kinase is highly expressed in the bronchial epithelial cells [17][18]. Therefore, we hypothesized that inhibition of Pim1 kinase reduces HRV replication in primary bronchial epithelial cells by augmenting the interferon-induced anti-viral response due to increased activity of the JAK-STAT signaling pathway.

In this study, we test our hypothesis by evaluating the effect of Pim1 kinase inhibition on viral replication in air-liquid interface cultures of primary bronchial epithelial cells derived from healthy individuals and severe asthmatic volunteers and assess the potential of pharmacological inhibition of Pim1 kinase activity as new therapeutic approach in virally induced asthma exacerbations.

## **Methods**

### **Cell cultures**

Bronchial brushings were obtained from subjects without or with asthma by fiber-optic bronchoscopy according to standard guidelines. All procedures were ethically approved by the Southampton and South West Hampshire Research Ethics Committees (REC no. 05/Q1702/165 and REC no. 09/H0504/109). Written informed consent was received from all volunteers. Detailed subject characteristics can be found in Table 1 in the online supplement.

Brushings were processed for primary bronchial epithelial cell (PBEC) culture in bronchial epithelium growth medium, as previously described [19]. When sub-confluent, cells were seeded on transwells and cultured on air-liquid interface (ALI) for 21 days. Fully differentiated ALI cultures were starved in basal medium and pre-treated with 5  $\mu$ M Pim1 inhibitor K00135 when indicated [20].

### **Human Rhinovirus-16**

Human rhinovirus-16 (ATCC® VR-283™, Teddington, UK) was amplified in H1HeLa cells as described previously [21]. The viral titer was determined by 50% tissue culture infective dose (TCID<sub>50</sub>) at  $1.76 \times 10^7$ /ml and multiplicity of infection (MOI) of 5 was used for all infections.

### **Viral infection**

ALI cultures of PBECs were apically infected for 6 hours at 33 °C, where after the cultures were washed apically twice with HBSS and the apical and basolateral medium, containing Pim1 inhibitor when indicated, was replaced. As controls, medium, UV-irradiated HRV-16 (1200 mJ/cm<sup>2</sup> for 50 minutes on ice) and Pim1 inhibitor alone were included.

## **RNA isolation, reverse transcription and RT-qPCR**

ALI cultures were harvested in TRIzol reagent (Invitrogen, Paisley, UK) and total RNA isolated following standard protocols. Contaminating DNA was enzymatically removed (DNA-free kit, Ambion, Austin, USA) and reverse transcription was performed with NanoScript reverse transcription kit (PrimerDesign Ltd, Southampton, UK).

mRNA expression levels were determined by RT-qPCR using 20 ng cDNA template and calculated relative to the average of the housekeeping genes GAPDH and UBC. A cloned synthetic DNA construct packaged at known copy number was used for determination of the viral copy numbers of HRV-16.

## **Analysis of viral particles and cytokines**

The release of viral particles in the supernatant was determined using TCID<sub>50</sub> assay [9]. The levels of IP-10 and RANTES in the apical supernatant were determined by ELISA (R&D Systems, Abingdon, UK), according to manufacturer's instructions with half of the recommended volumes used for every step.

## **Western Blotting**

ALI cultures were lysed in sample buffer and analyzed by SDS-PAGE. Antibodies for p-STAT-1 and pan-STAT-1 (9171 and 9172, Cell Signaling Technology Inc, Danvers, MA, USA) and  $\beta$ -Actin (A1978, Sigma-Aldrich, St. Louis, MO, USA) were used conform manufacturer's recommendations.

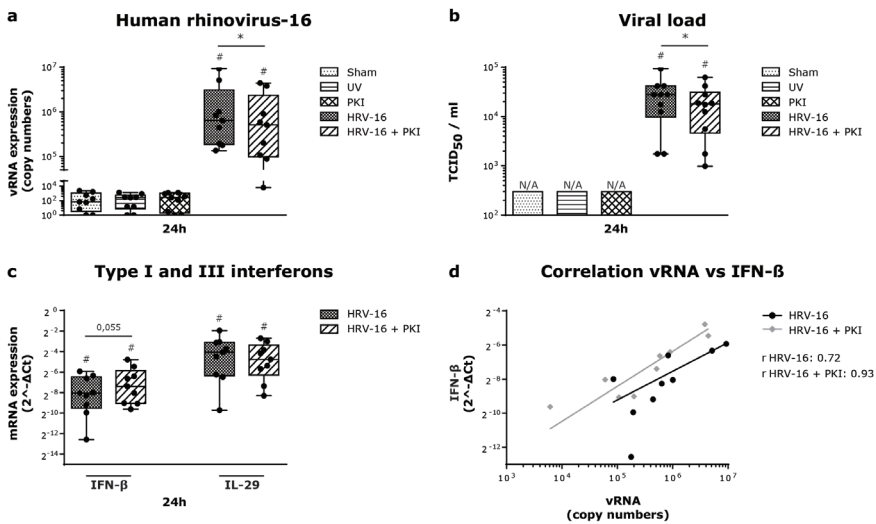
## **Statistical analysis**

The data were not normally distributed and were analyzed using the non-parametric Wilcoxon matched-pairs signed rank test.

## Results

### Inhibition of Pim1 kinase activity reduces viral replication in ALI cultures

Fully differentiated ALI cultures were infected with HRV-16 and significantly increased viral copy numbers were observed 24 hours after infection compared to the appropriate controls (Fig. 1a). Interestingly, concurrent inhibition of Pim1 kinase resulted in significantly decreased viral copy numbers compared to the cultures infected with HRV-16 alone.



**Figure 1: Inhibition of Pim1 kinase activity reduces viral replication in ALI cultures**

ALI cultures of PBECs from healthy volunteers, pre-treated with the Pim1 inhibitor (PKI) or control, were infected with HRV-16 at MOI of 5 for 6 hours at 33 °C and cultured until 24 after the start of infection. ALI cultures were analyzed for viral RNA copy numbers (a), viral load (b) and mRNA expression of IFN-β and IL-29 (c). Panel d shows the correlation between viral RNA copy number and mRNA expression of IFN-β. Median and range are shown. Statistical significance was tested with the paired two-sided Wilcoxon matches-pairs signed rank test and  $P < 0,05$ . Statistical significant differences  $P < 0,05$  compared to Sham-infected control are indicated by #.



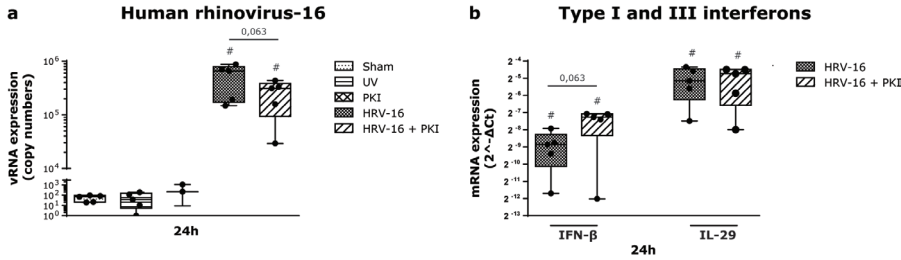
Subsequently, we analyzed the release of viral particles in the supernatant 24 hours after infection and consistent with the reduced viral copy numbers, inhibition of Pim1 kinase resulted in a decreased release of viral particles into the supernatant compared to the control cultures (Fig. 1b). Although the differences in response to viral infection were considerable between the individuals, the overall reduction in viral replication and release of viral particles was 20% and 35%, respectively (online supplement Fig. 1).

Analysis of mRNA expression of the Type I and III interferons 24 hours after infections revealed that infection with HRV-16 significantly induced the expression of IFN- $\beta$  and IL-29 compared to sham-infected control cultures (Fig 1c). While inhibition of Pim1 kinase resulted in a trend towards increased mRNA expression levels of IFN- $\beta$ , no differences in expression of IL-29 upon viral infection in the presence of the Pim1 kinase inhibitor were observed. However, even though interferon levels were not significantly increased, the levels of IFN- $\beta$  expression were enhanced relative to the viral load within the cell (Fig. 1d).

Taken together, these data suggest that pharmacological inhibition of Pim1 kinase activity reduces viral replication in ALI cultures of PBECs from healthy individuals in association with maintenance of a high level of IFN expression, even upon reduced viral replication.

### **Inhibition of Pim1 kinase activity reduces viral replication in ALI cultures of PBECs from severe asthmatic volunteers**

Since virally induced exacerbations are particularly problematic for asthmatic patients, we determined the effects of inhibition of Pim1 kinase activity on viral replication and anti-viral response in ALI cultures of PBECs from severe asthmatic volunteers. In the presence of the Pim1 kinase inhibitor, we observed a trend ( $p=0,063$ ) towards decreased viral copy numbers compared to the cultures infected in the absence of the Pim1 kinase inhibitor (Fig 2a).



**Figure 2: Inhibition of Pim1 kinase activity reduces viral replication in ALI cultures of PBECs from severe asthmatic volunteers**

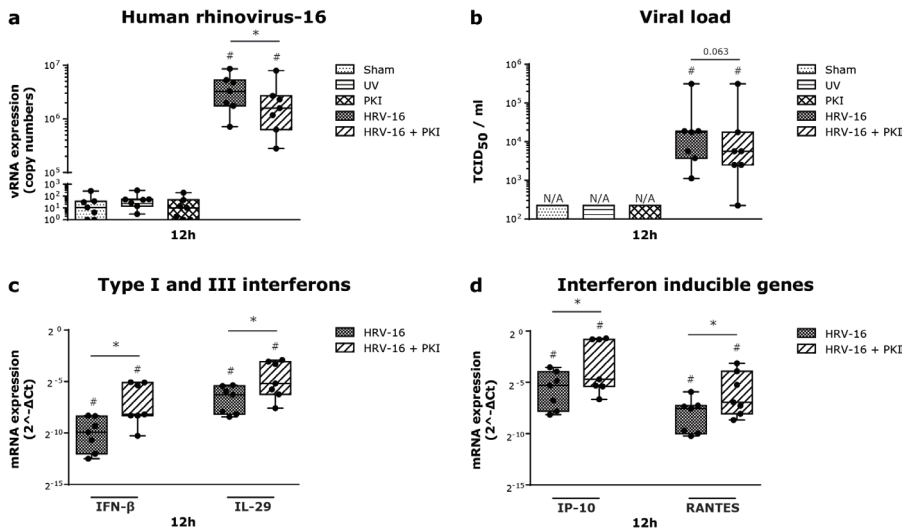
ALI cultures of PBECs from severe asthmatic volunteers, pre-treated with the Pim1 inhibitor (PKI) or control, were infected with HRV-16 at MOI of 5 for 6 hours at 33 °C and cultured until 24 hours after the start of infection. ALI cultures were analyzed for viral RNA copy numbers (a) and mRNA expression of IFN-β and IL-29 (b). Median and range are shown. Statistical significance was tested with the paired one-sided Wilcoxon matches-pairs signed rank test and  $P < 0,05$ . Statistical significant differences  $P < 0,05$  compared to Sham-infected control are indicated by #.

In line with our observations in the virally infected ALI cultures of PBECs from healthy individuals, infection with HRV-16 resulted in marked induction of IFN-β and IL-29 expression. As observed in the PBECs from healthy individuals 24 hours after infection, concurrent inhibition of Pim1 kinase resulted in a trend (0,063) towards increased IFN-β mRNA expression levels, while IL-29 mRNA expression was unaffected upon inhibition of Pim1 kinase activity (Fig. 2b). This effect was evident in 4 of 5 cases where treatment with the Pim1 inhibitor augmented IFN-β levels (online supplement Fig. 2a), while vRNA levels were reduced. In the subject where IFN-β was not increased, viral copy number was low and did not change in the presence of the inhibitor (online supplement Fig. 2b). These data suggest that, in most cases, inhibition of Pim1 kinase can also reduce viral replication in ALI cultures of PBECs from severe asthmatic volunteers with maintenance of the anti-viral response upon reduced viral replication.

## Inhibition of Pim1 kinase activity induces an early anti-viral response

To elucidate the mechanism behind the reduced viral replication upon inhibition of Pim1 kinase activity, we analyzed viral replication and the subsequent activation of the anti-viral response at earlier times after infection in ALI cultures of PBECS from healthy individuals.

Infection with HRV-16 resulted in highly increased viral copy numbers compared to control- treated ALI cultures 12 hours after infection, which was significantly reduced with 50% in the presence of the Pim1 kinase inhibitor (Fig. 3a and online supplement Fig.3a ). As shown in figure 3b, by this time, viral particles were detectable in the apical supernatant of the infected ALI cultures.



**Figure 3: Inhibition of Pim1 kinase activity induces an early anti-viral response**

ALI cultures of PBECS from healthy volunteers, pre-treated with the Pim1 inhibitor (PKI) or control, were infected with HRV-16 at MOI of 5 for 6 hours at 33 °C and cultured until 12 hours after the start of infection. The cultures were analyzed for viral RNA copy numbers (a), viral load (b), mRNA expression of IFN-β and IL-29 (c) and IP-10 and RANTES (d). Median and range are shown. Statistical significance was tested with the paired two-sided Wilcoxon matches-pairs signed rank test and  $P < 0,05$ . Statistical significant differences  $P < 0,05$  compared to Sham-infected control are indicated by #.

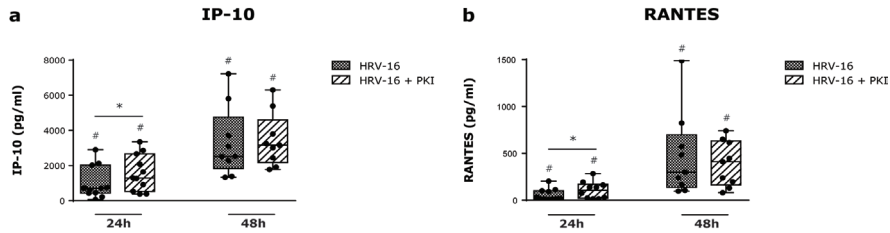
Here, we observed a trend ( $p = 0,063$ ) towards a decrease in the release of viral particles of approximately 70% in the presence of the Pim1 kinase inhibitor (online supplement Fig. 3b).

Analysis of the expression of IFN- $\beta$  and IL-29 showed strongly enhanced mRNA levels upon viral infection compared to mock-infected controls. Concurrent inhibition of Pim1 kinase activity further augmented the mRNA expression of both IFN- $\beta$  and IL-29 (Fig. 3c) and this effect of the inhibitor was dependent on viral replication (data not shown). Subsequent analysis of the mRNA expression of the interferon inducible genes IP-10 and RANTES showed a comparable effect: infection with HRV-16 induced the expression of IP-10 and RANTES, which was significantly enhanced in the presence of the Pim1 kinase inhibitor (Fig. 3d), but not by the inhibitor alone.

To summarize, these data demonstrate that inhibition of Pim1 kinase augments the early anti-viral response, resulting in enhanced expression of IFN- $\beta$  and IL-29 as well as their target genes IP-10 and RANTES, thereby leading to reduced viral replication.

### **Increased apical release of IP-10 and RANTES upon inhibition of Pim1 kinase activity**

To establish the mRNA expression data of IP-10 and RANTES seen 12 hours after infection, we measured the protein levels of these cytokines in the supernatant of the infected cultures. After 24 hours, inhibition of Pim1 kinase activity resulted in significantly increased levels of apically released IP-10 (Fig. 4a) and RANTES (Fig. 4b) compared to HRV-16 infection alone. The levels of IP-10 and RANTES were increased after 48 hours compared to the levels detected 24 hours after infection, but no differences could be observed upon inhibition of Pim1 kinase activity, reflecting the similar levels of IFN expression observed at 24 hours post infection (Fig. 1C). These data confirm that inhibition of Pim1 kinase activity results in enhanced anti-viral activity as evidenced by increased apical release of IP-10 and RANTES 24 hours after infection.

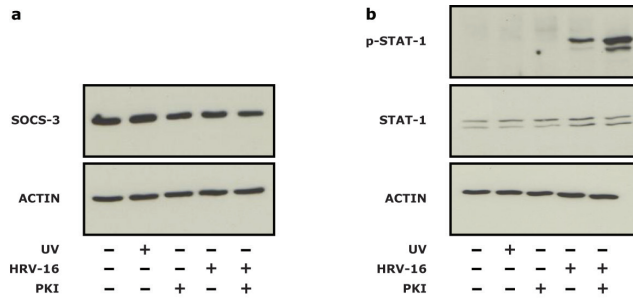


**Figure 4: Increased apically release of IP-10 and RANTES upon inhibition of Pim1 kinase activity**

ALI cultures of PBECs for healthy volunteers, pre-treated with the Pim1 inhibitor (PKI) or control, were infected with HRV-16 at MOI of 5 for 6 hours at 33 °C and cultured until 24 and 48 hours after the start of infection. The amount of apically released IP-10 and RANTES was determined with ELISA (a and b, respectively), 24 and 48 hours after infection. Median and range are shown. Statistical significance was tested with the paired two-sided Wilcoxon matches-pairs signed rank test and  $P < 0,05$ . Statistical significant differences  $P < 0,05$  compared to Sham-infected control are indicated by #.

### Inhibition of Pim1 kinase activity induces phosphorylation of STAT-1

To test if augmented proteosomal degradation of SOCS proteins upon inhibition of Pim1 kinase as postulated by Chen *et al* [16] was causative in the enhanced interferon signaling, we sought to measure the levels of SOCS-3 in the cell. Unfortunately, we were not able to detect significant differences in the levels of SOCS-3 proteins in cell lysates of ALI cultures 12 hours after infection (Fig. 5a). Therefore, we decided to test downstream activation of the interferon-signaling pathway and analyzed the phosphorylation of STAT-1. Phosphorylated STAT-1 was only detectable in virally infected ALI cultures, while total STAT-1 was detected at similar levels in all the cultures (Fig. 5b). Inhibition of Pim1 kinase activity resulted in strongly increased phosphorylation of STAT-1 compared to the HRV-16 infected cultures alone.



**Figure 5: Augmented phosphorylation of STAT-1 upon inhibition of Pim1 kinase activity**  
*ALI cultures of PBECs from healthy volunteers, pre-treated with the Pim1 inhibitor (PKI) or control, were infected with HRV-16 at MOI of 5 for 6 hours at 33 °C and cultured until 12 hours after the start of infection. Protein levels of SOCS-3 (Fig. 5a) and (phosphorylated) STAT-1 (Fig. 5b) were determined by Western blotting. Data are representative of 4 independent experiments.*

## Discussion

In this study, we assessed the potential of Pim1 kinase as novel target for therapeutic intervention in virally induced asthma exacerbations, using an HRV-16 infection model in PBECs cultured under ALI conditions. We demonstrated that 24 hours after infection, viral replication was reduced in ALI cultures of PBECs from healthy individuals and severe asthmatic volunteers treated with a pharmacological Pim1 kinase inhibitor. The reduced viral replication was accompanied by an augmented anti-viral inflammatory response characterized by increased gene expression of IFN- $\beta$ , IL-29, IP-10 and RANTES, 12 hours after infection and enhanced release of IP-10 and RANTES, 24 hours after infection. Western blot analysis of the phosphorylation status of STAT-1 revealed that inhibition of Pim1 kinase activity in virally infected ALI cultures of PBECs led to increased activation of STAT-1, 12 hours after infection, reflecting an increased type-I interferon signaling. These data suggest that Pim1 kinase suppresses the innate anti-viral response and that inhibition of its activity may thus offer a new therapeutic approach for virus-induced asthma exacerbations.

Asthmatic subjects suffer from exacerbations of their disease with one of the main triggers being respiratory virus infection [3]. The most commonly detected viruses are human rhinoviruses, of which more than 100 serotypes have been identified, precluding efficient design of vaccination strategies against HRVs [22]. While asthma has not shown to be a risk factor for the development of respiratory infections upon infection with HRV, the more frequent, more severe and longer lasting symptoms of lower-respiratory-tract infections observed in asthmatic patients compared to healthy individuals suggest an increased susceptibility towards rhinovirus infection [23]. The underlying mechanism for this enhanced susceptibility of asthma patients to rhinovirus infections has been associated with a deficiency of the innate immune response in the bronchial epithelium [9][10]. In the present study, we infected fully differentiated ALI cultures of PBECs from severe asthmatic

patients and healthy individuals with human rhinovirus. Exposing PBECs to an air interface in the presence of retinoic acid for 21 days results in a pseudostratified epithelium containing ciliated, goblet and basal cells, recapitulating the airway surface *in vivo* [24]. Although it has been shown before that differentiated PBECs are more resistant towards infection with HRV compared to the cells of monolayer cultures [25][26], we achieved unambiguous viral infection using a multiplicity of infection of 5, as assessed by viral replication and induction of the anti-viral responses in cultures from both healthy and severe asthmatic individuals. Although we did not observe significant differences in viral replication between differentiated cultures from healthy individuals and severe asthmatic volunteers, which is in line with previous studies [27][28], our studies were not powered to show such a difference.

We recently found that higher endogenous levels of TGF- $\beta$  in PBEC cultures from asthmatic volunteers augmented viral replication and suppressed IFN expression in association with increased gene expression levels of SOCS-1 and SOCS-3 [15]. SOCS proteins are negative regulators of interferon signaling by suppressing the intensity and duration of JAK-STAT activation [13][14]. In addition to transcriptional regulation, SOCS proteins can bind to Elongin BC E3 ligases, leading to their ubiquitin-mediated proteosomal degradation [29]. Of note, binding to Elongin BC E3 ligases can be prevented by phosphorylation of SOCS proteins by Pim kinases, thereby reducing their degradation and enhancing their suppressive activity towards JAK-STAT signaling [16]. Thus, the postulated mechanism of reduced viral replication upon inhibition of Pim1 kinase activity is enhanced proteosomal degradation of SOCS proteins leading to enhanced JAK-STAT activation and augmentation of the interferon-induced anti-viral response. Although we were not able to show decreased total levels of SOCS-3 protein in the presence of the Pim1 kinase inhibitor, this might be explained by relatively high basal levels of SOCS proteins impeding accurate quantification of differences upon viral infection and inhibition of Pim1 kinase activity. Nonetheless, we found



augmented phosphorylation of STAT-1 in virally infected ALI cultures treated with the Pim1 inhibitor, suggesting that inhibition of Pim1 kinase activity indeed resulted in enhanced activation of the JAK-STAT pathway. This activation was further established by increased expression levels of IFN- $\beta$  and IL-29, and increased expression of the interferon inducible genes IP-10 and RANTES at transcriptional and protein levels observed upon inhibition of Pim1 kinase activity in virally infected ALI cultures.

As observed with monolayer cultures pre-treated with IFN- $\beta$  to augment the anti-viral response [9][11], we observed reduced replication of HRV-16 in association with an augmented anti-viral response upon inhibition of Pim1 kinase activity using ALI cultures from either healthy individuals or severe asthmatic volunteers. These observations reveal that inhibition of Pim1 kinase activity can reduce viral replication, irrespective of the disease status, suggesting that inhibition of Pim1 kinase might be an interesting new therapeutic approach in the treatment of virus-induced asthma exacerbations.

Notwithstanding the promising results of inhibition of Pim1 kinase activity in reducing viral replication in ALI cultures of healthy individuals and severe asthmatic volunteers, we have to consider some limitations of our study. One limitation of our study is the use of pharmacological inhibition of Pim1 kinase activity. Although the Pim1 kinase inhibitor K00135 is highly specific and widely used in Pim1 kinase research [20][30][31], we cannot exclude the occurrence of off-target effects. However, the complexity of our *in vitro* model of ALI cultures of PBECs complicates the establishment of pharmacological inhibition of Pim1 kinase activity with for example siRNA constructs to knock-down the activity at transcriptional level. A second limitation of our study is the single use of HRV-16 to induce viral infections in bronchial epithelial cells. Next to HRV, influenza and

respiratory syncytial viruses (RSV) are commonly detected in exacerbations and it would be worthwhile to establish if inhibition of Pim1 kinase activity can be used as treatment in asthma exacerbations induced by viruses of different origin [1].

In summary, our data showed that inhibition of Pim1 kinase activity reduces viral replication in ALI cultures of PBECs from healthy individuals and severe asthmatic volunteers by enhancing the activity of STAT-1, resulting in an augmented interferon-induced anti-viral response. Further studies focusing on the mechanism of the enhanced interferon response upon inhibition of Pim1 kinase activity should establish our findings and reveal the clinical relevance of inhibition of Pim1 kinase activity as novel therapeutic treatment in virally induced asthma exacerbations.

### *Acknowledgments*

The authors would like to thank Juerg Schwaller for providing the Pim1 inhibitor K00135 and Robert Ridley and Graham Berreen for the technical assistance with the ALI PBEC cultures.

### *Grants*

This work was supported by ERS Fellowship LTRF 2013-2135 to Maaïke de Vries, a shared University of Groningen and University of Southampton PhD studentship (GUIDE PhD studentship to Maaïke de Vries) and Medical Research Council (UK) grant number G0900453.

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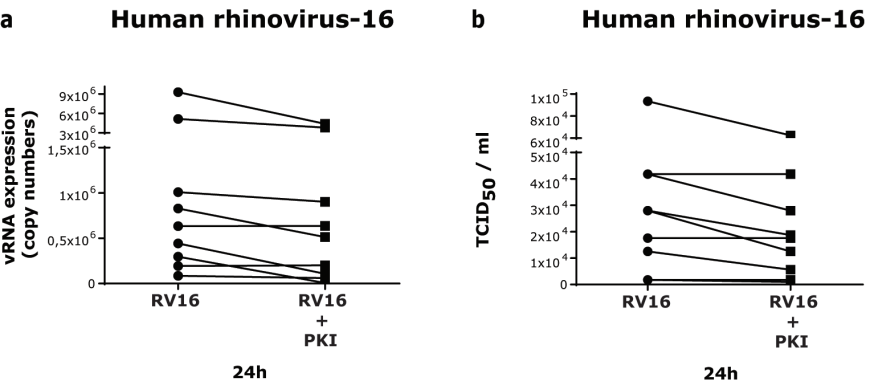
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Supplementary data

**Supplement Table 1:** Summary of clinical and physiological characteristics of subjects used to study the effects of inhibition of Pim1 kinase activity on viral replication and anti-viral response upon infection with HRV-16.

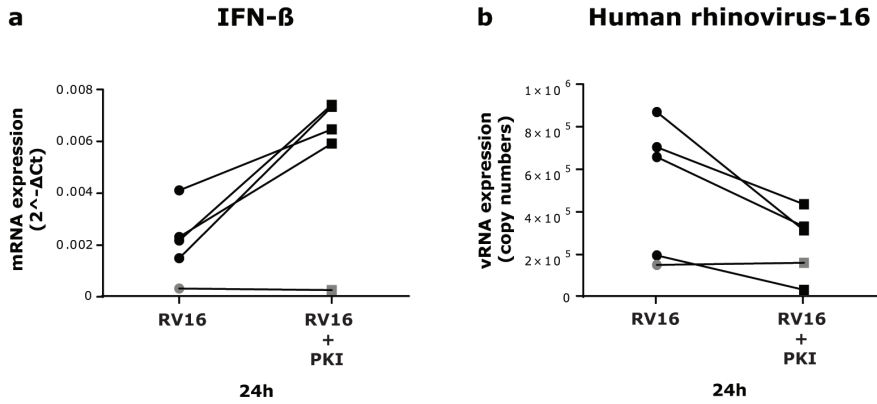
	Age (y)	Sex, male/female	FEV <sub>1</sub> (% predicted)	Inhaled corticosteroids yes/no
Nonasthmatic	36 (range, 19 - 70)	11/4	100,5 (range, 83 - 120,06)	0/15
Asthma	38 (range, 24 - 62)	2/3	78,2 (range, 41 - 112)	5/0

Asthma was diagnosed by physicians according to the British Thoracic Society guidelines. Subjects without asthma were healthy subjects selected from the University of Southampton volunteers database. They had no history of asthma and no underlying disease. All subjects were recruited after ethical approval and informed consent.



**Supplement Figure 1: Effects of inhibition of Pim1 kinase activity on viral copy numbers and release of viral particles in HRV-16 infected ALI cultures of PBECs from healthy individuals**

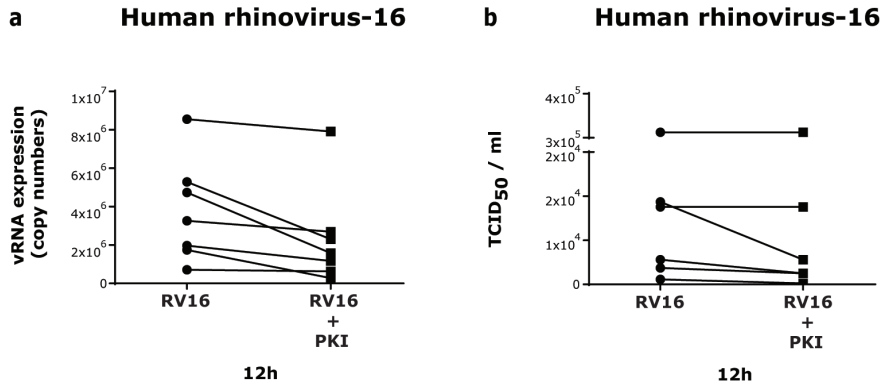
ALI cultures of PBECs from healthy individuals were infected with HRV-16 at MOI of 5 for 6 hours at 33 °C in the absence or presence of the Pim1 kinase inhibitor (PKI) and cultured until 24 hours after the start of infection. Viral RNA copy number (a) and release of viral particles (b) are shown for each donor in the absence or presence of PKI. Data are representative of 9 and 10 independent experiments, respectively.



**Supplement Figure 2: Effects of inhibition of Pim1 kinase activity on mRNA expression of IFN-β and viral RNA copy numbers in HRV-16 infected ALI cultures of PBECs from severe asthmatic volunteers**

ALI cultures of PBECs from severe asthmatic volunteers were infected with HRV-16 at MOI of 5 for 6 hours at 33 °C in the absence or presence of the Pim1 kinase inhibitor (PKI) and cultured until 24 hours after the start of infection. mRNA expression of IFN-β (a) and viral RNA copy numbers (b) are shown for each donor in the absence and presence of the Pim1 kinase inhibitor. Data are representative of 5 independent experiments.





**Supplement Figure 3: Effects of inhibition of Pim1 kinase activity on viral copy numbers and release of viral particles in HRV-16 infected ALI cultures of PBECs from healthy individuals**

ALI cultures of PBECs from healthy individuals were infected with HRV-16 at MOI of 5 for 6 hours at 33 °C in the absence or presence of the Pim1 kinase inhibitor (PKI) and cultured until 12 hours after the start of infection. Viral RNA copy number (a) and release of viral particles (b) are shown for each donor in the absence or presence of PKI. Data are representative of 7 independent experiments.



